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Unique Myeloid Leukemias in Young Children with Down Syndrome: Cell Origin, Association with Hematopoietic Microenvironment and Leukemogenesis

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1. Introduction

Patients with Down syndrome (DS) are at 10- to 36-fold higher risk of developing leukemia (Roy et al., 2009). In children with DS aged 4 years or older, acute lymphoblastic leukemia (ALL) is the predominant type of leukemia just as it is in the general pediatric population, whereas acute myeloid leukemia (AML) is more common than ALL in patients with DS less than 4 years of age. Interestingly, acute megakaryoblastic leukemia (AMKL), a rare subtype of AML in non-DS patients, comprises 62-86% of AML cases in children with DS (Hitzler, 2007; Roy et al., 2009), which will be referred to as AMKL-DS hereafter. Furthermore, hematological abnormalities that are indistinguishable from AMKL-DS occur in about 10% of neonates with DS but spontaneously disappear within several months of life. This disorder has been given a variety of names, including transient leukemia (TL), transient myeloproliferative disorder (TMD) and transient abnormal myelopoiesis (TAM). In 20-30% of patients with TL, AMKL-DS develops later through the stage of myelodysplastic syndrome (MDS) within 4 years. These disorders, namely, TL, MDS and AMKL-DS, in young children with DS have many unique features and had been considered a disease entity that was called "Myeloid leukemias of Down syndrome", then later renamed "Myeloid proliferations related to Down syndrome" in the current World Health Organization (WHO) Classification published in 2008. This review summarizes recent data on clinical, cellular and molecular biological aspects of these myeloid neoplasms with special reference to the origin of neoplastic cells, the organs where they arise and multistep model of leukemogenesis.

2. Transient leukemia (TL)

2.1 Clinical features

TL is a disorder of neonates with DS, with median age at diagnosis being 7 days (range, 1-65 days) (Massey et al., 2006). Clinical manifestations in symptomatic cases include hepatosplenomegaly, effusions, bleeding and skin rash, but there are no overt signs of symptoms related to TL in other cases. TL is usually found as a result of a routine medical

checkup or incidental blood examination performed because of another unrelated illness. The patients remain well and the disease gradually disappears within the first 3 months of life in most cases without any therapy and the prognosis is generally good. However, severe life-threatening complications occur in approximately 15% of patients (Hitzler, 2007). These include two major forms; 1) liver dysfunction caused by infiltration of leukemic blasts and liver fibrosis, leading to progressive obstructive jaundice and liver failure; and 2) cardiopulmonary disease, manifesting as hydrops-like symptoms, including pulmonary edema, pleural or pericardial effusions and ascites (Zipursky, 2003). Leukemic blasts are usually present in the effusions. Other serious complications include hyperviscosity due to massive leukocytosis and hepatosplenomegaly that impairs spontaneous respiration. These patients with severe complications have benefitted from treatment with low-dose cytarabine (Ara-C) (Al-Kasim et al., 2002; Dormann et al., 2004; Klusmann et al., 2008). Furthermore, in 20 to 30% of patients with TL that has spontaneously regressed, AMKL-DS later develops within 4 years of life. In rare cases, however, complete regression of TL does not occur and regrowth of blasts with acquired additional cytogenetic abnormalities directly leads to AMKL-DS.

Laboratory investigations usually demonstrate marked leukocytosis with varying proportions of circulating blasts (Massey et al., 2006). The bone marrow contains increased numbers of blasts but, interestingly, the ratio of blasts in the marrow is often lower than that in the peripheral blood, a peculiar finding for AML because the marrow is usually packed with blasts when a large number of blasts are present in the blood. This phenomenon is considered due to the fetal liver origin of TL, as described below in more detail, and the marrow is only secondarily involved by the disease process. The bone marrow may also contain dysplastic mature megakaryocytes and exhibit features similar to those of MDS that precedes the onset of AMKL-DS (Zipursky et al., 1999).

TL may occur in utero and cause intrauterine fetal death as a result of non-immune hydrops fetalis and cardiac dysfunction due to leukemic cell infiltration into the pericardial or cardiac muscular tissues (Zipursky et al., 1996; Heald et al., 2007; Ishigaki et al., 2011) or visceral fibrosis (Becroft & Zwi, 1990; Ruchelli et al., 1991; Becroft, 1993). Prenatal diagnosis of TL can be made by ultrasonographical detection of hydrops or hepatosplenomegaly followed by chromosomal analysis and hematological examination of fetal blood obtained by umbilical cord centesis (Gray et al., 1986; Zerres et al., 1990; Foucar et al., 1992; Smrcek et al., 2001; Robertson et al., 2003). Accurate estimation of the frequency of TL in fetuses and neonates is difficult because stillbirths with TL may be missed due to the low autopsy rate of stillbirths, or fetuses with TL may spontaneously recover in utero and because TL in neonates without complications may disappear without being noticed. It is roughly estimated that TL occurs in about 20% of patients with DS, including about half of those dying in utero (Zipursky, 2003), but the true incidence of TL needs to be clarified based on prospective population-based studies. TL also occurs in phenotypically normal individuals with trisomy 21 mosaicism (Brodeur et al., 1980; Kalousek & Chan, 1987). In these patients, leukemic blasts always have trisomy 21, indicating that trisomy 21 is an essential prerequisite for TL.

2.2 Characteristics of leukemic blasts

Light microscopically, the blasts of TL may be morphologically undifferentiated (Fig. 1a) or exhibit features of megakaryoblasts with cytoplasmic blebs, similar to AMKL-DS blasts (Fig. 1b), or micromegakaryocytes. Although myeloperoxidase (MPO) is negative, flow cytometric cell surface marker analysis of blasts demonstrates expression of antigens related to multiple

hematopoietic cell lineages, including megakaryocytes (CD41, CD42b, CD61), granulocytes (CD13, CD33, CD38), erythroid cells (glycophorin, CD71), stem cells (CD34, CD117) and, in addition, certain characteristic lymphoid markers (CD7 and CD56) (Yumura-Yagi et al., 1992; Langebrake et al., 2005; Massey et al., 2006). Electron microscopic examination demonstrates that the leukemic cells in TL are more heterogeneous than those in AMKL-DS, exhibiting features of megakaryoblasts with varying degree of megakaryocytic differentiation (Fig. 2a), granulocytic precursors (Fig. 2b), including basophils, and erythroid cells (Bessho et al., 1988; Eguchi et al., 1989; Eguchi et al., 1992). The megakaryocytic nature of blasts can be demonstrated by the presence of platelet specific granules (α granules), platelet demarcation membrane and/or positive reaction for platelet peroxidase (PPO) that is present in the perinuclear space and rough endoplasmic reticulum but not in the Golgi apparatus and α granules (Fig. 2a). Some blasts may possess peculiar cytoplasmic granules with internal membranous structures (Fig. 2a, inset), which are called θ granules because of their resemblance to the Greek letter theta (θ) (Bessho et al., 1988; Eguchi et al., 1989; Eguchi et al., 1992). These structures are known to be present in immature precursors of not only megakaryocytic, but also erythroid (Coulombel et al., 1987) and mast cell/basophil (Parkin et al., 1980) lineages. Extreme basophilia in a phenotypically normal newborn with TL, whose leukemic cells showed a chromosome 21 abnormality, has been reported (Worth et al., 1999). These data indicate that the blasts of TL are derived from multipotential hematopoietic progenitors, not restricted to megakaryocytic lineage, and are consistent with the multilineage differentiation potential of TL blasts seen *in vitro* as described below (section 2.4).

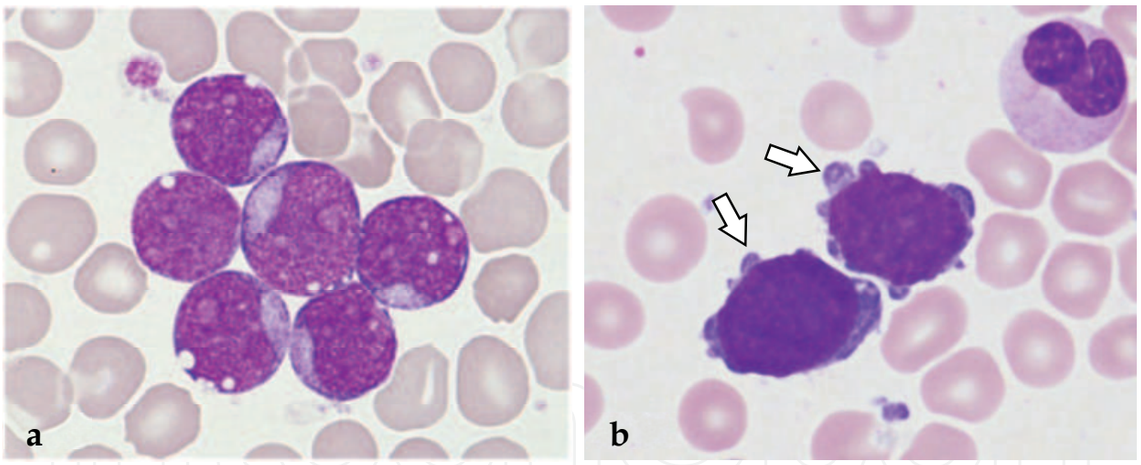


Fig. 1. Morphology of leukemia cells in TL and AMKL-DS. (a) Blasts of TL in the peripheral blood with primitive morphology. (b) Blasts of AMKL-DS in the bone marrow. Note the presence of cytoplasmic bleb (arrow), indicating megakaryoblastic nature.

By utilizing allele-specific polymorphism of genomic markers that reside on the X chromosome and inactivation pattern of one of the X chromosomes in female cells, it has been shown that the blasts of TL are monoclonal populations of cells in the majority of cases (Kurahashi et al., 1991; Miyashita et al., 1991; Massey et al., 2006), indicating that TL is a neoplastic disorder and not a reactive leukemoid reaction, although later works with *GATA1* gene analysis demonstrated that TL in some cases may contain oligoclonal populations of neoplastic cells (see section 4.2). Spontaneous regression of TL does not rule

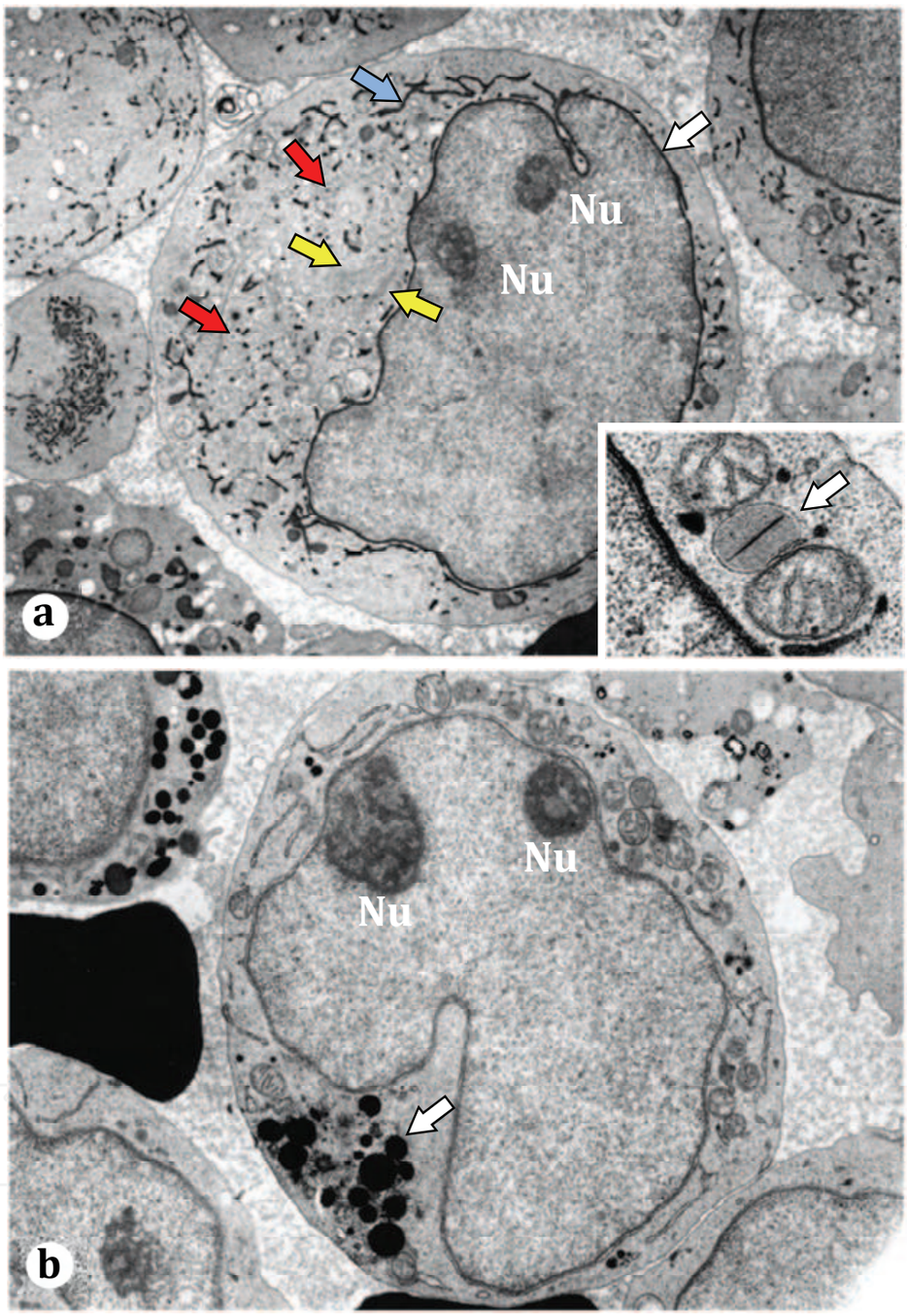


Fig. 2. Electron microscopic appearance of blasts in TL. (a) PPO reaction is positive in the perinuclear space (white arrow) and rough endoplasmic reticulum (blue arrow) but not in α granules (red arrow) and the Golgi apparatus (yellow arrow), indicating megakaryoblastic nature. (Inset) Higher magnification of a θ granule (arrow). (b) A few MPO-positive granules are present (arrow) but other organelles are negative for MPO in this cells, indicating abnormal, minimal myeloid differentiation. Nu, nucleolus.

out the leukemic nature of this disease, since 1) spontaneous regression can be seen in other unquestionable malignant neoplasms, such as neuroblastoma in infants; 2) blasts that are indistinguishable from AMKL-DS blasts appear in the blood and infiltrate in the tissues; and 3) TL can be a fatal disorder in severe cases due to tissue infiltration of blasts in major organs, such as the liver and heart. Based on these data, in addition to other cellular and molecular biological characteristics as described below, TL is now considered a special type of preleukemia or a very unusual form of leukemia with self-limiting growth potential.

2.3 Chromosomal analysis

Chromosomal abnormalities seen in blasts of TL usually include only trisomy 21 in both patients with DS and those with trisomy 21 mosaicism and no other chromosomal abnormalities are present in most cases. This is an important point in the differential diagnosis of TL from AMKL-DS, which usually shows a variety of clonal chromosomal abnormalities (Hayashi et al., 1988). In rare cases, however, abnormalities other than trisomy 21 are found in TL blasts, including additional chromosomes 12 and 14, deletion of a chromosome, der(X;15)(p10;q10), an extra C chromosome and polyploidy with 57 chromosomes (Zipursky, 2003). These abnormalities usually disappear along with spontaneous remission of TL and are usually absent in the blasts of AMKL-DS that has later arisen in the same patients and developed other chromosomal abnormalities. However, in some cases, they may be present in subsequent AMKL-DS blasts (Kitoh et al., 2009), evidence that AMKL-DS develops in some of the clones of TL blasts.

2.4 Differentiation capability of leukemic blasts

When TL blasts are cultured in the presence of hematopoietic growth stimulants, such as phytohemagglutinin-stimulated leukocyte conditioned medium (PHA-LCM) or recombinant hematopoietic growth factors, mature or maturing hematopoietic cells of various lineages appear, including basophils, neutrophils, eosinophils, monocytes and erythroid cells (Suda et al., 1987). However, it was uncertain whether these cells were all derived from TL blasts rather than coexisting normal hematopoietic progenitors in the samples examined. We have recently demonstrated that TL blasts are capable of differentiating into basophil/mast cell lineages when cultured in the presence of interleukin-3, stem cell factor (SCF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) (Fig. 3a), and into megakaryocytes in the presence of thrombopoietin (TPO) (Fig. 3b), by demonstrating that the differentiated cells that appeared after culture carried the same *GATA1* mutations as the TL blasts did before culture (Miyauchi et al., 2010) (see section 4.2). Consistent with these *in vitro* data, massive increase of basophils in the peripheral blood of a patient with TL has been reported (Worth et al., 1999) and another case demonstrating pericardial effusion containing predominantly basophils has been described (Zipursky et al., 1997). We recently reported pathological findings on autopsy of a stillbirth with TL, in which numerous megakaryoblasts and dysplastic megakaryocytes were present in the liver and blood vessels, whereas leukemic blasts infiltrating into the peripheral tissues, including pericardium, expressed MPO (Ishigaki et al., 2011). These findings are consistent with the *in vitro* data described above and indicate that blasts in TL are not simply megakaryoblasts but derived from more primitive hematopoietic progenitors that are capable of differentiating into several myeloid lineages *in vivo*, possibly depending on the hematopoietic microenvironment. The differentiation capability of TL blasts into mature

blood cells is unique for AML and might be somehow associated with the spontaneous remission of this disorder. Although TL blasts express some markers of the erythroid lineage and, in fact, erythroblasts at various stages of differentiation appeared in culture of TL blasts in the presence of erythropoietin and SCF in combination, these cells expressed full-length GATA1 but not aberrant GATA1s protein (see section 4.2) and, therefore, it was shown that these differentiated erythroid cells were derived from coexisting normal erythroid progenitors (Miyauchi et al., 2010).

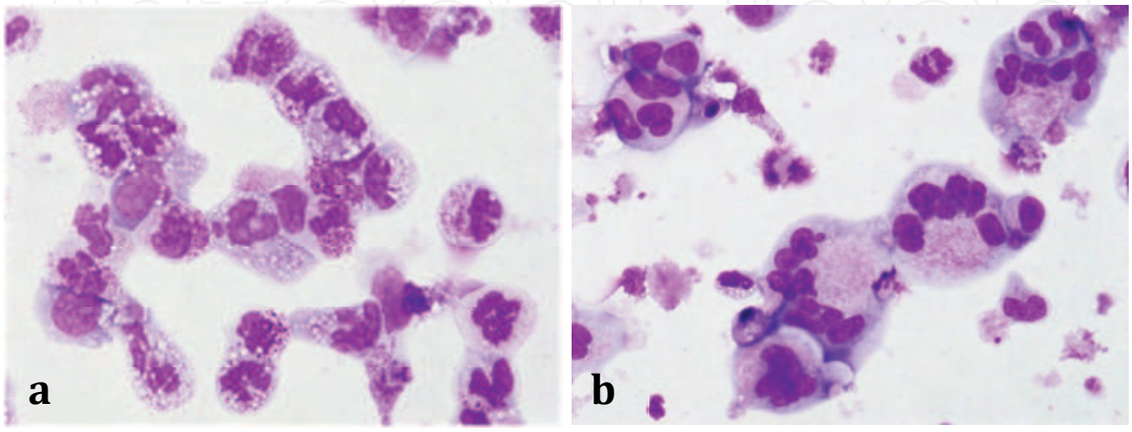


Fig. 3. Morphology of TL blasts after culture in the presence of hematopoietic growth factors. (a) Basophils that appeared in culture with GM-CSF. (b) Mature megakaryocytes that appeared in culture with TPO.

2.5 Origin of leukemic progenitors and association with hematopoietic microenvironment

Although most patients with TL show a favorable prognosis, serious complications develop in some cases as described above. While myelofibrosis is one of the characteristic features of AMKL-DS (Fig. 4a, b), autopsy cases of patients with TL have demonstrated that these patients often exhibit unusual diffuse sinusoidal liver fibrosis (Fig. 4c, d), but not myelofibrosis (Becroft & Zwi, 1990; Ruchelli et al., 1991; Miyauchi et al., 1992; Yagihashi et al., 1995; Schwab et al., 1998; Shiozawa et al., 2004). It has been shown that leukemic blasts in AMKL produce cytokines, including platelet-derived growth factor (PDGF), platelet factor 4 and transforming growth factor β (TGFβ) that stimulate fibroblasts in the bone marrow causing myelofibrosis (Breton-Gorius et al., 1982; Roberts et al., 1986; Sunami et al., 1987; Terui et al., 1990). Since blasts in TL have features similar to those of megakaryoblasts in AMKL-DS and TL is a disorder of neonates and fetuses, it appears that TL is a very unusual form of neoplasia originating from the fetal liver, the major organ of hematopoiesis during the fetal stage, and that leukemic blasts that arise in the fetal liver produce cytokines that stimulate fibroblasts to induce liver fibrosis in the same manner as myelofibrosis in AMKL-DS. Proliferation of dysplastic megakaryocytes and blasts, including megakaryoblasts, in the liver has been shown in autopsy cases of fetuses with TL (Ruchelli et al., 1991; Becroft, 1993; Ishigaki et al., 2011) and production of TGFβ by TL blasts in the liver has been immunohistochemically demonstrated (Arai et al., 1999). The presence of unique hematopoietic progenitors originating from the yolk sac and fetal liver that are sensitive to

GATA1s transgene to cause hyperproliferation of megakaryocytes only during certain fetal developmental stages has been demonstrated by experiments using knock-in mice (Li et al., 2005) (see section 4.2), indicating that these cells are likely the target of leukemic progenitors in TL.

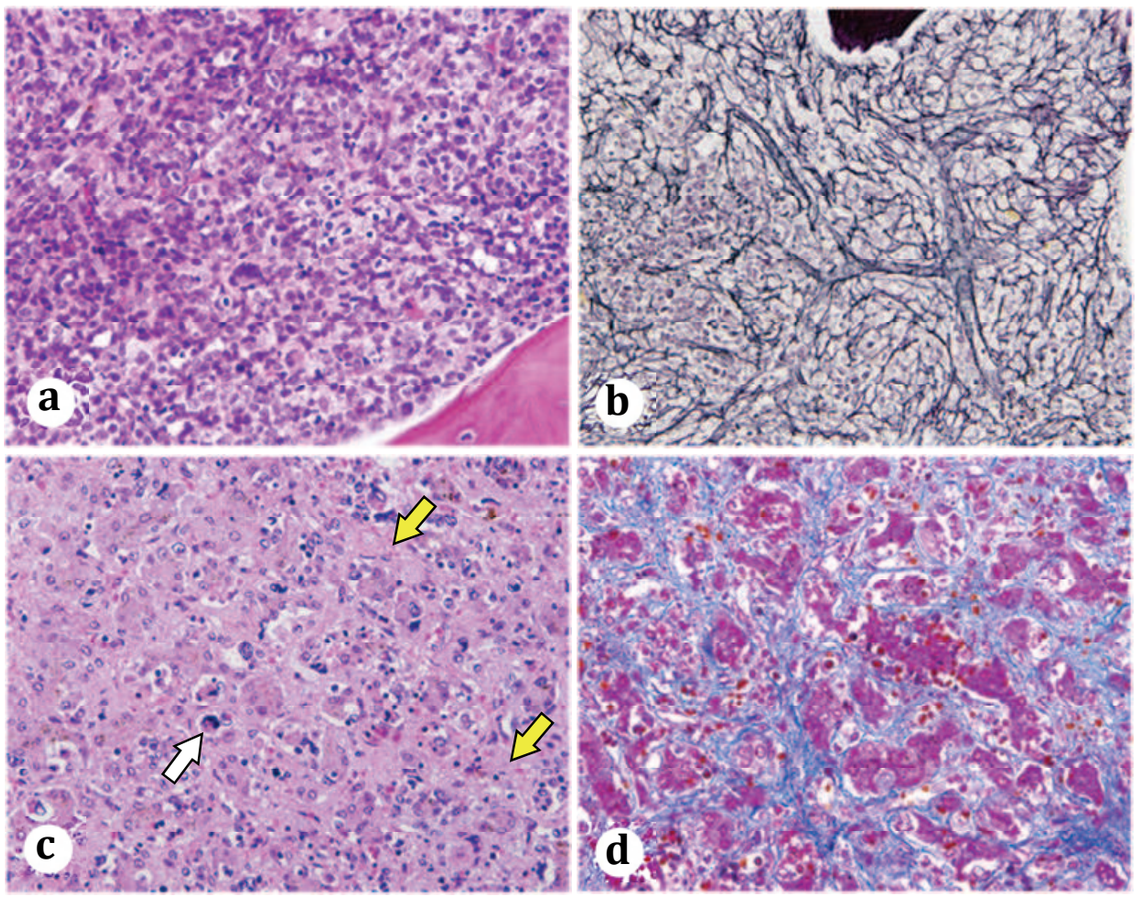


Fig. 4. Histopathology of the bone marrow and liver in patients with AMKL-DS and TL. (a) The bone marrow in a patient with AMK-DS is packed by monotonous blasts, a finding consistent with acute leukemia (H-E stain). (b) Silver impregnation staining of the marrow demonstrates increase of reticulin fibers (myelofibrosis), which is one of the characteristic findings of AMKL. (c) The liver in a patient with TL after regression (H-E stain). Perisinusoidal fibrosis is present (yellow arrow) accompanied by marked distortion of hepatic cords. Atypical megakaryocytes are also seen (white arrow). (d) Azan stain clearly demonstrates perisinusoidal fibrosis of the liver (stained in blue).

2.6 Mechanism of spontaneous remission

The mechanism underlying spontaneous remission of TL is largely unknown, but several plausible explanations have been proposed. First, if the target cells of origin in TL are fetal hematopoietic progenitors of limited lifespan, the growth and differentiation of which are governed by genetic mechanisms controlling fetal hematopoiesis, a developmental switch in genetic control from fetal to adult hematopoiesis after birth may cease the proliferation of

leukemic blasts ("intrinsic theory") (Ahmed et al., 2004; Li et al., 2005). Second, if TL is an unusual form of leukemia occurring in the fetal liver, but not in the bone marrow, and the growth of blasts in TL is dependent exclusively on the microenvironment of the fetal liver, a transition of the major site of hematopoiesis from the liver to the bone marrow after birth and cessation of the hepatic hematopoiesis would prevent the growth of TL blasts and cause regression of the disease ("environmental theory") (Miyauchi et al., 1992; Gamis & Hilden, 2002; Ahmed et al., 2004). Other possible mechanisms that may also explain spontaneous remission of TL include the capability of differentiation of TL blasts (Suda et al., 1987). As described above, blasts in TL can differentiate into mature blood cells of at least several lineages *in vitro* and *in vivo*. According to changes in environmental factors that control fetal and adult hematopoiesis after birth, differentiation of TL blasts might be induced, leading to cessation of the growth of TL blasts. Self-induced apoptosis of TL blasts possibly mediated by increased expression of superoxide dismutase, which has been linked to increased apoptosis in DS models and the gene of which is located on chromosome 21, has also been proposed as a cause of spontaneous regression (Taub et al., 2004). Further studies are required to clarify which hypotheses, alone or in combination, are responsible or whether other mechanisms participate in the spontaneous remission of TL.

3. Megakaryoblastic leukemia in Down syndrome (AMKL-DS)

3.1 Clinical features

Patients with DS are susceptible to AMKL, which comprises about 62-86% of AML in DS patients (Hitzler, 2007; Roy et al., 2009). Since AMKL is an infrequent subtype of AML in non-DS patients, the incidence of AMKL-DS compared to that of AMKL in non-DS patients has been estimated to be about 500 times higher. AML in older patients with DS is only rarely AMKL, does not demonstrate *GATA1* mutations (see section 4.2) and is a disease distinct from AMKL-DS.

AMKL-DS has many unique features compared with AMKL in non-DS patients. First, it often occurs in patients with a history of TL within the first 4 years of life after TL has resolved or, rarely, during the process of incomplete remission of TL, although cases of AMKL-DS without preceding TL have also been documented (Ahmed et al., 2004). Second, while AMKL in non-DS is clinically aggressive and the prognosis is poor, AMKL-DS shows a very high remission rate and favorable prognosis, with survival probability ranging between 70 and 90%, in response to chemotherapy (Hitzler, 2007). The cause of favorable prognosis of AMKL-DS is thought, at least in part, to be due to the high sensitivity of leukemic blasts to chemotherapy such as Ara-C. Third, AMKL-DS in 20-60% of patients is preceded by a prolonged period of cytopenia (usually several months to even years of thrombocytopenia) accompanied by proliferation of dysplastic megakaryocytes in the bone marrow, which corresponds to the MDS phase, before the onset of AMKL-DS (Zipursky et al., 1994). This preceding MDS phase is not present in other forms of AMKL in non-DS patients and is unique to AMKL-DS.

3.2 Common and distinct features of leukemic blasts in AMKL-DS and TL

Besides the difference in age of onset, there are many other differences as well as common features between AMKL-DS and TL. Blasts in AMKL-DS and TL exhibit great similarity in cytological characteristics, including morphology, cytochemistry and cell surface antigen expression. Blasts in AMKL-DS exhibit megakaryoblastic morphology in both light and

electron microscopic observation likewise the case of TL (Fig. 1b). Flow cytometric analysis of blasts in AMKL-DS also shows almost identical antigen expression to that of TL blasts, except for a somewhat lower expression of CD34 and CD56 in AMKL-DS than in TL. In contrast to the blasts of TL, in which trisomy 21 is the sole chromosomal abnormality in most cases, blasts in AMKL-DS usually show a variety of chromosomal abnormalities besides constitutional trisomy 21, with additional chromosome 8 or 21 being most frequent. Although hepatic fibrosis is often seen in TL patients with severe liver dysfunction, fibrosis of the bone marrow (myelofibrosis) is one of the characteristic features of AMKL-DS (Fig. 4a, b), indicating that AMKL arises in the bone marrow whereas TL may arise in the fetal liver, organs that are the major sites of hematopoiesis after and before birth, respectively. In contrast to the benign and self-limiting clinical course of TL, AMKL-DS is potentially a lethal disorder, which does not exhibit spontaneous remission and requires chemotherapy, although the cure rate and prognosis is better than those of AMKL in non-DS patients. *GATA1* gene mutations are present in nearly all patients with AMKL-DS as in the case of TL (see section 4.2).

4. GATA1 and its role in leukemogenesis

4.1 Structure and function of GATA1

GATA1 is a member of the six GATA family of zinc-finger transcription factors (GATA1 to GATA6), which share a highly conserved zinc finger domain that recognizes the consensus nucleotide sequence motif (A/T)GATA(A/G) (Cantor, 2005). GATA1 plays important roles in hematopoiesis in a lineage-specific manner for erythroblasts, megakaryocytes, mast cells and eosinophils. Mutations of the *GATA1* gene, which resides on the X chromosome at Xp11.23, have been shown to play a critical role in leukemogenesis of DS-related myeloid leukemias. The full length GATA1 protein (molecular weight: approximately 50kD) contains three well characterized domains; two zinc finger domains (N-terminal and C-terminal zinc fingers) and a transcriptional activation domain at the N-terminal portion of the protein (Fig. 5). The C-terminal zinc finger is required for DNA binding, while the N-terminal zinc finger stabilizes this interaction and mediates interactions with a cofactor Friend of GATA1 (FOG1) (Tsang et al., 1997). The full-length GATA1 protein is produced by translation from methionine at codon 1 (Met1) on exon 2 (n.b., exon 1 is not coding) (Fig. 5). In some type of cells, another shorter isoform of GATA1 (molecular weight: approximately 40kD), referred to as GATA1s, is physiologically produced in a much smaller amount by alternative splicing from Met84 on exon 3 of the full GATA1 mRNA (Calligaris et al., 1995) (Fig. 5). GATA1s lacks N-terminal activation domain and has reduced transactivation potential, but it retains two zinc fingers and, therefore, can bind DNA and interact with FOG1. GATA1s is produced in a variety of cell lines and normal fetal liver and is thought to be important for embryonic development.

4.2 *GATA1* gene mutations in TL and AMKL-DS

Mutations affecting the *GATA1* gene in patients were first reported by Wechsler et al. (2002) exclusively in leukemic cells of AMKL-DS, and subsequently many groups of investigators reported *GATA1* mutations in nearly all patients with TL, MDS and AMKL in DS patients (Greene et al., 2003; Groet et al., 2003; Hitzler et al., 2003; Mundschau et al., 2003; Rainis et al., 2003; Xu et al., 2003). In these reports, the mutations have not been detected either in AMKL of non-DS patients or in other types of leukemias in DS patients, indicating that *GATA1* mutations are specific to TL, MDS and AMKL in DS patients. The mutations include a variety of abnormalities, such as nonsense/missense point mutations, deletions, insertions, or splice site mutations, which are so diverse as to be clone-specific markers. However, most

of these abnormalities are clustered within exon 2 or less commonly in exon 3 (Fig. 5), resulting in loss of the first initiation codon (Met1) or disruption of the normal reading frame and introduction of a premature termination codon. Since *GATA1* gene is located on the X chromosome, when the allele harboring mutated *GATA1* is active, the other allele with wild-type *GATA1* is inactivated by methylation in female cells. Therefore, only the mutant *GATA1* is expressed in both male and female patients. The mutated *GATA1* gene invariably fails to produce full-length GATA1 and generates only the GATA1s isoform lacking N-terminal transcriptional activation domain using an alternative downstream initiator codon Met84 on exon 3 (Fig. 5). The other mutation, that is, splice site mutation that occurs in the boundary between exon 2 and intron 2 disrupts mRNA splicing and generates only shorter splice variant mRNA (GATA1s mRNA), in which exon 2 is skipped, and, consequently, only short isoform of GATA1, equivalent to GATA1s, is produced (Rainis et al., 2003) (Fig. 5).

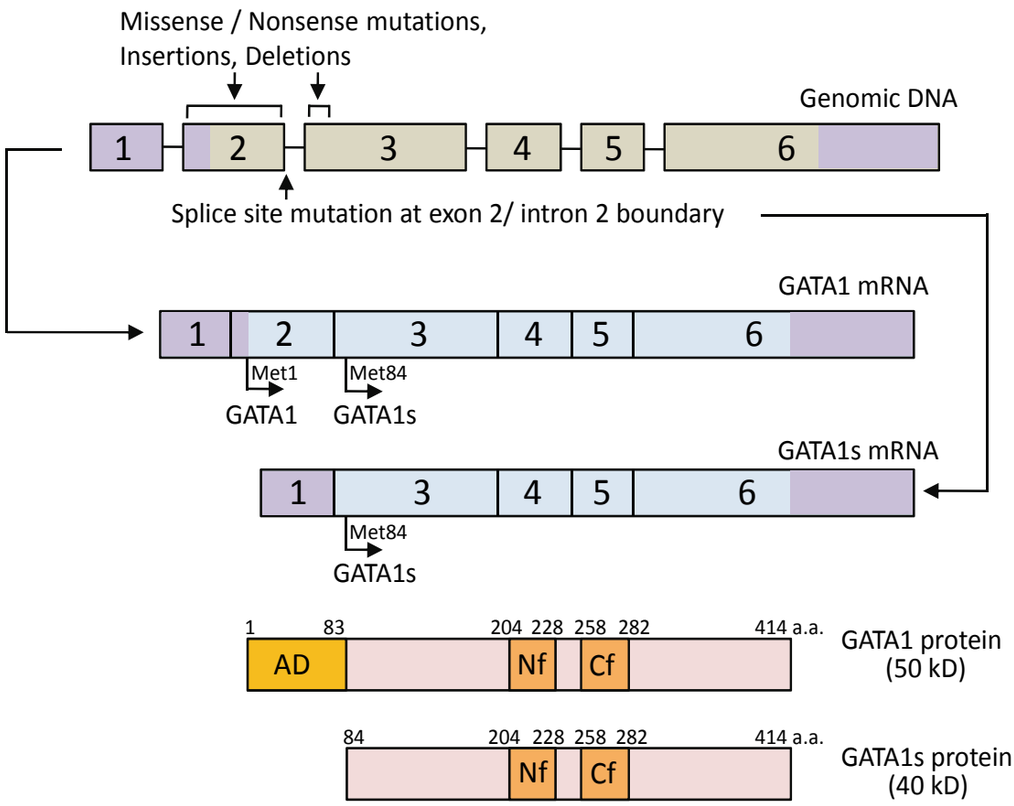


Fig. 5. Mutations of the *GATA1* gene in TL and AMKL-DS. Genomic DNA encoding *GATA1* consists of 6 exons (depicted as boxes and numbered from 1 to 6). The area colored in purple are 5′ and 3′ non-coding regions. Physiologically, normal full-length GATA1 protein is produced by translation from Met1 on exon 2, whereas GATA1s protein from Met84 on exon 3 of full GATA1 mRNA by alternative splicing. Most *GATA1* mutations occur on exon 2 or at exon2/intron2 boundary and all result in the production of only GATA1s, but the former by alternative splicing using downstream initiation codon Met84 whereas the latter by producing GATA1s mRNA and translation from it. Abbreviations: AD, transactivation domain; Nf, N-terminal zinc finger; Cf, C-terminal zinc finger; a.a., amino acid.

GATA1 mutations are not detected at the stage of remission in either TL or AMKL-DS (Rainis et al., 2003; Ahmed et al., 2004), indicating that the mutations are acquired somatic events. Accumulated data suggest that *GATA1* mutations occur in utero: 1) since TL is a disorder of neonates and almost certainly arises in utero, *GATA1* mutations that are present in almost all cases of TL should also occur in utero; 2) exactly the same mutations have been identified in the blasts of AMKL-DS in identical twins (Rainis et al., 2003), indicating that abnormal cells with *GATA1* mutations arose in one of the twins during the fetal stage and transferred to the other twin via anastomosing blood vessels in the placenta; 3) the same *GATA1* mutations have been detected in the neonatal blood spots of patients with AMKL-DS, who did not have clinically overt antecedent TL (Ahmed et al., 2004); 4) *GATA1* mutations have been detected in neonatal blood spots from 2 of 21 otherwise healthy DS children but not from non-DS cord blood samples (Ahmed et al., 2004); and 5) *GATA1* mutations have been detected in genomic DNA from 2 of 9 fetal liver and 2 of 5 infantile bone marrow autopsy specimens from patients with DS (Taub et al., 2004). Thus, *GATA1* mutations appear to occur in utero, if not in all cases, at relatively high frequency and specifically in patients with DS.

Although TL and AMKL-DS are typically disorders consisting of monoclonal population of cells carrying a single type of *GATA1* mutation, there have been reports of cases of TL and AMKL-DS with multiple independent clones with different *GATA1* mutations in single patients (Ahmed et al., 2004; Groet et al., 2005; Miyauchi et al., 2010). In one of four AMKL-DS patients with multiple *GATA1* mutations, neonatal blood spot showed 3 independent mutations but only one of these was present in AMKL-DS blasts (Ahmed et al., 2004), indicating that AMKL-DS had evolved from one of these oligoclonal cells with different *GATA1* mutations that had occurred in utero. Similarly, evolution of AMKL-DS from one of the oligoclonal populations of TL blasts with different chromosomal abnormalities after regression of TL has been demonstrated (Kitoh et al., 2009). While identical mutations between the blasts in TL and AMKL-DS that occurred later in the same patient have been reported by several investigators (Wechsler et al., 2002; Hitzler et al., 2003; Rainis et al., 2003; Shimada et al., 2004), different mutations between the blasts of TL and subsequent AMKL in the same patient have also been reported (Xu et al., 2006b; Kanegane et al., 2007). In the patient of Xu et al., however, although predominant clones of TL and AMKL-DS were different, a minor clone of TL with *GATA1* mutation identical to that of AMKL-DS was present. Taken together, it appears highly likely that AMKL-DS evolves from a minor clone (or multiple clones in some cases) of TL blasts that have persisted after regression, or from one or multiple silent clones of cells with *GATA1* mutations that have not expanded to develop into clinically detectable TL but survived persistently in the body, possibly in the bone marrow.

4.3 The role of GATA1 and GATA1s in TL and AMKL-DS

Since high expression of GATA1s and abrogation of full-length GATA1 is the invariable result of the *GATA1* gene mutations, increased GATA1s and/or loss of full-length GATA1 protein must play a crucial role in leukemogenesis in both TL and AMKL-DS, possibly through altered interaction with their partner proteins. Since GATA1s has a reduced transactivation potential due to the lack of an N-terminal activation domain but can bind DNA and interact with the cofactor FOG1 via zinc finger domains, its role as a dominant negative protein, which fails to activate or repress the function of proteins that are normally regulated by GATA1, has been proposed (Gurbuxani et al., 2004). Alternatively, since

normal GATA1 protein binds to RUNX1, which is an important megakaryopoietic regulator encoded by the *RUNX1* gene that resides on chromosome 21, and the binding site has been shown to be located at the N- and C-terminal portions of GATA1 (Elagib et al., 2003), GATA1s lacking an N-terminus may cause abnormal growth and/or differentiation of neoplastic cells involving the megakaryocytic lineage through defective biding to RUNX1. However, the site on GATA1 that binds to RUNX1 is controversial; it has been shown that GATA1 interacts with RUNX1 through zinc fingers, but not the N-terminal portion (Waltzer et al., 2003), and that GATA1s of all patient samples examined bound to RUNX1 through zinc finger domains (Xu et al., 2006a). The role of RUNX1 in leukemogenesis of DS-related leukemias needs to be further determined. We have recently shown that the expression level of GATA1s decreases during the process of growth factor-induced differentiation of TL blasts *in vitro*, indicating that GATA1s may act as a repressor of the GATA1-related proteins that induce differentiation and that the protein level of GATA1s changes depending on the cellular circumferential conditions and may be a key factor that influences the growth and differentiation of TL blasts (Miyauchi et al., 2010). Consistent with this finding, it has been described that expression of GATA1s is decreased *in vivo* in murine mature megakaryocytes carrying a *GATA1* mutation that results in the production of GATA1s equivalent to the protein products of *GATA1* mutations in DS-related leukemias (Majewski et al., 2006). Kanezaki et al. (2010) showed that protein levels of GATA1s in TL blasts differ depending on the sites of mutations and its quantitative differences are significantly associated with patient prognosis and the risk of developing AMKL-DS. These data suggest that protein levels of GATA1s may also be important in the biology of DS-related leukemic cells.

Experiments using mouse models with various *GATA1* gene alterations have shown that the presence of full-length GATA1 is crucial in fetal development, particularly for megakaryocyte and erythroid lineages. Complete absence of GATA1 in male mice (GATA1 null mice) results in embryonic lethality due to severe anemia (Fujiwara et al., 1996). Reduced expression of GATA1 in GATA1.05 mice, in which GATA1 expression is reduced to less than 5% of the normal level, also causes embryonic lethality in male hemizygous and female homozygous mice, whereas female heterozygous mice survive the fetal stage but develop hematological abnormalities similar to MDS and die prematurely (Takahashi et al., 1997; Takahashi et al., 1998). Another mouse strain (lineage-selective GATA1 knock-out mouse), in which GATA1 expression is virtually absent from megakaryocytes, exhibits marked thrombocytopenia and morphologically abnormal megakaryocytes with impaired cytoplasmic maturation proliferate and accumulate in the spleen and bone marrow (Shivdasani et al., 1997). Abnormal proliferation of megakaryocyte/erythroid progenitors in GATA1-deficient murine embryonic stem cell-derived hematopoietic cultures has also been described (Stachura et al., 2006). Thus, loss of normal full-length GATA1 might also play an important role in leukemogenesis of TL and AMKL-DS. However, the extent to which the leukemic phenotype is due to the loss of full-length GATA1 versus high expression of GATA1s remains to be explored.

5. Multistep model of myeloid leukemogenesis in children with DS

Based upon the data described above, a multistep model of leukemogenesis of myeloid leukemias in children with DS has been proposed (Ahmed et al., 2004; Gurbuxani et al., 2004; Cantor, 2005; Hitzler & Zipursky, 2005; Hitzler, 2007; Roy et al., 2009; Zwaan et al., 2010) (Fig. 6). TL and AMKL-DS are disorders closely associated with DS, and although they

rarely occur in phenotypically normal patients with trisomy 21 mosaicism, trisomy 21 is always present in all leukemic cells of such patients, indicating that trisomy 21 must be the prerequisite of these disorders. Several mouse models of DS have been developed, which can be used to explore the important dose-dependent genes that are involved in DS-specific disorders and study the pathology of model mice in comparison with human DS patients. These model mice include Ts65DN, Ts1Cje (Carmichael et al., 2009) and Tc1 (Alford et al., 2010) mouse strains, that are trisomic for 143, 94 and 269 gene orthologues, respectively, of 324 recognized genes on human chromosome 21, with the Tc1 strain representing the most complete model of human DS generated to date. These mice all show macrocytic anemia and some of these mouse strains show increased numbers of megakaryocytic and erythroid precursors in the adult spleen and develop myeloproliferative disorder in adults. However, none of these mouse strains develop TL and AMKL, indicating that additional genetic abnormalities are required to cause leukemia. In humans as well, trisomy 21 itself has been shown to disturb fetal liver, but not bone marrow, hematopoiesis, enhance production of megakaryocyte/erythroid progenitors (MEPs), which may be susceptible to acquisition of other genetic abnormalities, and predispose these cells to DS-related leukemias (Chou et al., 2008; De Vita et al., 2008; Tunstall-Pedoe et al., 2008). These data support the model that the genes on chromosome 21 play essential roles in the development of these disorders and trisomy 21 is the first step of myeloid leukemogenesis in DS.

The genes *RUNX1* (alternatively called *AML1*), *BACH1*, *ETS2* and *ERG*, all of which are located on chromosome 21 and are associated with megakaryopoiesis, have been suggested to be the candidate genes involved in leukemogenesis of TL and AMKL-DS (Ahmed et al., 2004; Gurbuxani et al., 2004; Cantor, 2005; Hitzler & Zipursky, 2005; Osato & Ito, 2005; Hitzler, 2007; Roy et al., 2009; Zwaan et al., 2010). Translocations and point mutations of *RUNX1*, leading to loss of function or haploinsufficiency (namely, reduced expression) of RUNX1, have been detected in a variety of human leukemias and are thought to be involved in leukemogenesis (Yamashita et al., 2005). Alternatively, increased dosage of expression of these genes due to trisomy 21 has also been suggested to be another mechanism of leukemogenesis (Yanagida et al., 2005). However, expression levels of RUNX1 are not necessarily increased in all tissues in patients with DS or DS model mice (Osato & Ito, 2005); therefore, the above theory regarding RUNX1 requires further verification. ERG has been shown to be expressed in AMKL-DS, strongly cooperate with GATA1s and immortalize megakaryocytic progenitors (Salek-Ardakani et al., 2009), indicating that ERG in trisomy 21 may play a role in the development of DS-related leukemias. It has been shown recently that miR-125b-2, a microRNA (miRNA) that is located on chromosome 21, is upregulated in the samples of patients with TL and AMKL-DS and that its overexpression stimulates proliferation and self-renewal of megakaryocytic progenitors and MEPs and accentuates proproliferative effects of GATA1s on MEPs in murine fetal liver, indicating that miRNAs related to chromosome 21 might also participate in leukemogenesis in patients with DS (Klusmann et al., 2010). The role of the genes or miRNAs on chromosome 21 in DS-related leukemogenesis would be the major concerns in future studies.

The second step is most likely the acquisition of *GATA1* mutations in hematopoietic progenitor cells (Fig. 6), since it has been demonstrated that 1) *GATA1* mutations are present exclusively in the blasts of TL and AMKL-DS in nearly all patients; 2) these are acquired somatic mutations; and 3) these mutations occur in utero. Furthermore, it has been reported that a germline splicing mutation of *GATA1*, leading to synthesis of only GATA1s, caused anemia and neutropenia but not leukemia in seven affected males from two generations of a

family, indicating that a *GATA1* mutation alone may cause hematological abnormalities but requires trisomy 21 to cause TL or AMKL (Hollanda et al., 2006). However, it is of note that N-terminally truncated *GATA1* mutant in a non-DS-model mouse caused massive accumulation of megakaryocytes in the fetal liver that spontaneously resolved after birth, similar to TL in humans, indicating that a *GATA1* mutation alone may cause TL-like megakaryocytic hyperproliferation in mice (Shimizu et al., 2009).

The target cells of *GATA1* mutations are likely to be embryonic or fetal primitive hematopoietic progenitor cells with multilineage differentiation potential. With the acquisition of *GATA1* mutations, these cells would give rise to oligoclonal or monoclonal populations of neoplastic cells in the fetal liver (Fig. 6). The large clone(s) may develop into TL and cause hepatic fibrosis and dysfunction through the production of collagen-stimulating cytokines or infiltrate into the tissue, causing cardiac failure or hydrops fetalis. *GATA1* mutations in cooperation with trisomy 21 may cause TL but they should be insufficient to immortalize the blasts, leading to spontaneous remission before or after birth through unknown mechanism(s).

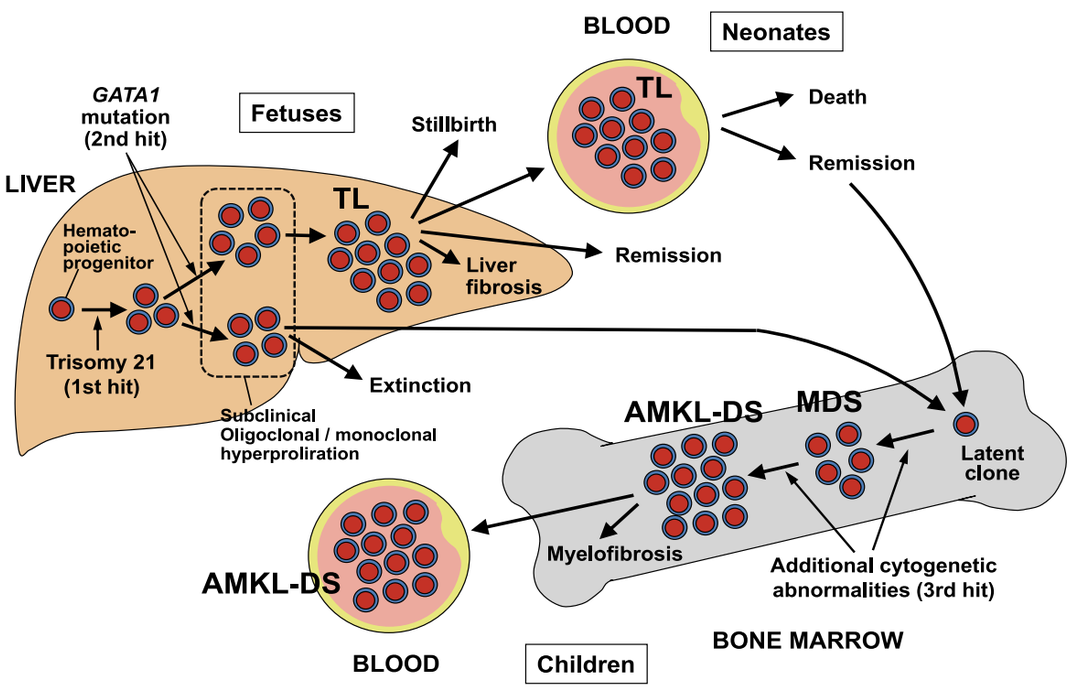


Fig. 6. Multistep model of myeloid leukemogenesis in young children with DS.

It is plausible that minor clones of residual TL blasts or clinically silent neoplastic hematopoietic progenitor cells that have obtained *GATA1* mutations but have not caused TL could survive latently during postnatal life and, with the acquisition of additional genetic abnormalities as the third step, would cause AMKL-DS through the stage of MDS in the bone marrow (Fig. 6). Although trisomy 8, altered telomerase activity (Holt et al., 2002), and mutations in several genes, including *TP53* (Malkin et al., 2000; Hirose et al., 2003), *KIT*, *MPL*

and *FLT3* (Malinge et al., 2008), have been identified in some patients with AMKL-DS, genetic abnormalities that cause evolution of AMKL-DS from those latent clones of cells with *GATA1* mutations are largely unknown. Several recent studies have shown activating mutations of the Janus kinase 3 (*JAK3)* gene, which encodes for a non-receptor tyrosine kinase, in some patients with TL and AMKL-DS (De Vita et al., 2007; Kiyoi et al., 2007; Klusmann et al., 2007), but these mutations were present in both TL and AMKL-DS and, therefore, are not likely involved in disease progression from TL to AMKL-DS. Concerning the evolution of AMKL-DS, Kanezaki et al. (2010) have recently demonstrated that the expression levels of GATA1s protein are associated with the type of *GATA1* mutations in TL; low expression of GATA1s is caused by mutations introducing premature termination codon (PTC) at the 5′ side of exon 2 or after codon 84 on exon 3 whereas high expression of GATA1 is associated with mutations causing loss of the first methionine, splicing error or introduction of PTC at the 3′ side of exon 2, and that GATA1s low mutations in TL are significantly associated with higher risk of developing AMKL-DS. The mechanism by which AMKL-DS evolves from minor clones of cells with *GATA1* mutations is currently one of the main areas of research in DS-associated leukemias.

6. Conclusion

Young children with DS are predisposed to unusual leukemias of myeloid origin, namely, TL and AMKL-DS. In contrast to the transient nature of the former, which places it in a category of preleukemia or "unusual" leukemia, the latter is an authentic leukemia leading to a lethal outcome unless treated. Nevertheless, these disorders are closely linked to each other by many common cellular morphological as well as cytogenetic features, including trisomy 21 and *GATA1* gene mutations, and share a distinct pathogenetic basis. Recent investigations have demonstrated much of molecular basis of these disorders and contributed to the proposal of an attractive model of a stepwise leukemogenic process of these disorders. This new model is expected to suggest many directions for future studies on not only DS-related leukemias but also pediatric leukemias in general.

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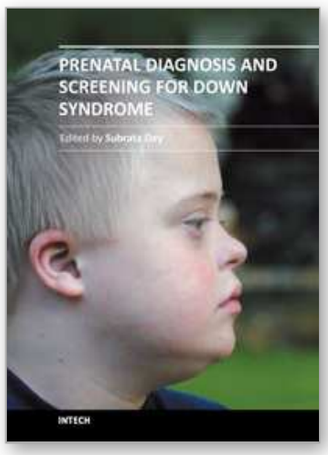
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Prenatal Diagnosis and Screening for Down Syndrome
Edited by Prof. Subrata Dey

ISBN 978-953-307-355-2
Hard cover, 232 pages
Publisher InTech
Published online 17, August, 2011
Published in print edition August, 2011

This book provides a concise yet comprehensive source of current information on Down syndrome. Research workers, scientists, medical graduates and paediatricians will find it an excellent source for reference and review. This book focuses on exciting areas of research on prenatal diagnosis - Down syndrome screening after assisted reproduction techniques, noninvasive techniques, genetic counselling and ethical issues. Whilst aimed primarily at research worker on Down syndrome, we hope that the appeal of this book will extend beyond the narrow confines of academic interest and be of interest to a wider audience, especially parents and relatives of Down syndrome patients.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jun Miyauchi (2011). Unique Myeloid Leukemias in Young Children with Down Syndrome: Cell Origin, Association with Hematopoietic Microenvironment and Leukemogenesis, Prenatal Diagnosis and Screening for Down Syndrome, Prof. Subrata Dey (Ed.), ISBN: 978-953-307-355-2, InTech, Available from: http://www.intechopen.com/books/prenatal-diagnosis-and-screening-for-down-syndrome/unique-myeloid-leukemias-in-young-children-with-down-syndrome-cell-origin-association-with-hematopoi

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